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Platelets Influence Vascularized Organ Transplants from Start to Finish

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Abstract

This review relates the basic functions of platelets to specific aspects of organ allograft rejection. Platelet activation can occur in the donor or recipient before transplantation as well as during antibody- and cell-mediated rejection. Biopsies taken during organ procurement from cadaver donors have documented that activated platelets are attached to vascular endothelial cells or leukocytes. In addition, many patients waiting for transplants have activated platelets due to the diseases that lead to organ failure or as a result of interventions used to support patients before and during transplantation. The contribution of platelets to hyperacute rejection of both allografts and xenografts is well recognized. Intravascular aggregates of platelets can also be prominent in experimental and clinical transplants that undergo acute antibody or cell-mediated rejection. In acute rejection, platelets can recruit mononuclear cells by secretion of chemokines. After contact, monocytes, macrophages and T cells interact with platelets through receptor/ligand pairs, including P-selectin/PSGL-1 and CD40/CD154. There is a potential for therapy to inhibit platelet mediated immune stimulation, but it is counterbalanced by the need to maintain coagulation in the perioperative period.

Keywords

Chemokines; endothelial cells; ischemia; macrophage; platelet; rejection; T cell

Introduction

Platelets are a ubiquitous blood cell with well-established roles in thrombosis and the acute response to trauma. However, they are increasingly being recognized as having a fundamental role in the initiation of adaptive immune responses, including alloimmune responses. The basic function of platelets and the mechanisms promoting interactions between platelets and endothelial cells, macrophages and lymphocytes were recently summarized in a previous mini review (1). In this review, we will relate these basic functions of platelets to organ allograft rejection.

Few reports have specifically addressed platelets in organ transplantation. Nevertheless, platelets have been associated with significant morphological and functional findings before and after transplantation, and are clearly part of the recipient response to a transplanted organ.

Platelets are often underestimated on routine histological examination because their absence of a nucleus precludes detection with hematoxylin and their small amount of cytoplasm minimizes staining with eosin. However, immunohistology for various adhesion molecules expressed on activated platelets, such as various glycoproteins (e.g. GPIIb; CD41 or GPIIIa; CD61), P-selectin (CD62P) or von Willebrand factor (vWf), often reveal an unexpectedly large number of platelets attached to vascular endothelial cells or leukocytes.

Once viewed as an entirely stochastic process, platelet localization to the graft is increasingly understood as a controlled process appropriate to its potent immunostimulatory effects. Platelets have been shown to express functional formyl peptide receptors and exhibit formyl peptide gradient-dependent chemotaxis *in vitro* (2). While formyl peptides are typically viewed as exclusive to prokaryotic organisms, it is interesting to note that human mitochondria express formyl peptides and that lysed mitochondria can promote platelet chemotaxis that can be inhibited with monoclonal antibodies specific for platelet formyl peptide receptors (2). This suggests that a fundamental mechanism of platelet localization to a tissue is related to cell death and resultant leak of mitochondrial contents.

Activated platelets have been documented independent from clot in organs at the time of procurement and reperfusion, as well as during antibody- and cell-mediated rejection. Thus, platelets have potential to influence all aspects of transplant biology.

Platelet activation by trauma and brain death

The potential impact of platelets on deceased donor grafts begins with the direct effects of donor trauma. Traumatic exposure of subendothelial matrix proteins leads to platelet activation and degranulation. Alpha granules are the most abundant secretory granule in platelets and their degranulation liberates many cytokines, vasoactive chemicals and adhesion molecules relevant to generation of immune responses (3,4). Although isolated degranulation evokes potent but appropriate local effects, catastrophic injury such as that seen in fatal brain trauma may evoke a degree of platelet activation that has systemic influences. Indeed, these may contribute to a proinflammatory environment prior to organ procurement.

One substantial effect of degranulation is to facilitate direct platelet-mediated activation of circulating leukocytes. Major trauma results in conformational changes of GPIIb/IIIa, and expression of P-selectin on platelets (5,6). Constitutive surface GPIIb/IIIa facilitates platelet binding to fibrinogen, vWf, fibronectin and vitronectin which is enhanced by activation-induced conformational changes. P-selectin exposed through degranulation promotes interaction with neutrophils, monocytes and lymphocytes, resulting in conjugates of platelets and leukocytes circulating subsequent to trauma. In addition, activated platelets form microparticles.

There is increasing evidence that platelet-derived microparticles have significant effects in inflammation that are dependent on their surface composition and content. Platelet microparticles are formed by blebs from the surface plasma membrane (ectocytotic microparticles) or fusion of intracellular multivesicular endosomes with the cell surface (exosomes). Microparticles are generated by most platelet agonists including, collagen, thrombin, ADP and epinephrine. These microparticles express many proteins not on resting platelets (7,8). The surface composition of microparticles varies with the agonist, but usually include P-selectin, GPIb, GPIIb/IIIa and JAM-A (junctional adhesion molecule). Jacoby et al. (5) have reported that platelet-derived microparticles are increased more in the circulation of patients with than without brain injury. These microparticles alter endothelial function by transcellular delivery of arachidonic acid products and chemokines (7,8).

Activation of platelets consequent to trauma is consistent with several reports of increased numbers of activated platelets deposited in organs from deceased donors. Cywes et al. (9) used GPIb as a marker to assess platelet adhesion to endothelial cells in wedge biopsies from 30 human livers at the start of organ procurement and 1 h after reperfusion in the recipient. Almost one-third of the biopsies had significant numbers of adherent platelets at the time of procurement that were not appreciated by routine histological stains. The subsequent biopsy indicated that ischemia-reperfusion increased the amount of platelet adhesion.

Recipient platelet activation pretransplantation and with graft reperfusion

Many patients waiting for transplants have activated platelets as a result of the diseases that lead to organ failure. For example, arteriosclerosis, a major underlying disease in many transplant-relevant conditions, is associated with increased platelet activation (10). In addition, many interventions used to support patients before and during transplantation also can stimulate platelet function. Platelets are activated by bioincompatible surfaces and artificial shear stresses in dialysis machines, ventricular assist devices and cardiopulmonary bypass machines (11, 12). Moreover, unlike healthy endothelial cells, artificial surfaces do not express or secrete mediators that regulate activation of platelets. As a result, transplanted kidneys, hearts and lungs are met by platelets expressing P-selectin and platelet-leukocyte conjugates immediately upon reperfusion. These activated platelets from the recipient may contribute to the platelets observed in early biopsies of transplants from deceased donors.

Platelets are known to lodge in recently reperfused organs. Immunohistological studies for vWF, CD41 (GP IIb) and P-selectin have demonstrated activated platelets more frequently in the vasculature of deceased donor kidneys and livers compared to live donor organs (13,14). Colombat et al. found a correlation between intravascular aggregates of P-selectin-positive platelets and indices of primary graft failure within the first 3 days after lung transplantation (15).

Although P-selectin is expressed by endothelial cells as well as platelets (Table 1), the P-selectin contributed by platelets seems essential. In order to determine the relative contribution of P-selectin from platelets versus endothelial cells, Ley et al. created chimeras that expressed either P-selectin in platelets or endothelial cells by bone marrow transplantation between wild-type and P-selectin-deficient mice. Expression of P-selectin on platelets, but surprisingly not on endothelial cells, was sufficient to cause neutrophil infiltration into the cortical-medullary junction following ischemia reperfusion injury to kidneys (16). Khandoga et al. (17) have reported that interactions between activated platelets, CD4⁺ T cells and sinusoidal endothelial cells contribute to postischemic liver injury in mice. Their experiments show that this interaction is promoted by P-selectin and CD40 on platelets binding to their corresponding ligands, PSGL-1 and CD154 on CD4₊ T cells. Similar mechanisms are active in cardiac grafts (18), and lung grafts (19).

Platelet activation factor (PAF) is another potent mediator of inflammation that is expressed by a variety of cells, including both platelets and endothelial cells (Table 1). PAF causes acute vascular alterations in part through platelet aggregation and endothelial cell retraction. A contribution of PAF to intestinal ischemia-reperfusion injury has been demonstrated with PAF receptor knockout mice, and several studies have demonstrated beneficial effects of PAF antagonists on preservation of lungs (20).

Robson et al. (21) have emphasized the importance of dysregulated endothelial control of nucleotides released by platelets during ischemia-reperfusion injury. Activation of platelets through ADP is regulated by CD39, an ectonucleotidase expressed on the membranes of normal vascular endothelium. CD39 hydrolyses ADP released from dense granules of activated platelets. However, vascular CD39 activity is lost under conditions of inflammatory or oxidant

stress, including ischemia-reperfusion injury and rejection processes thus potentially augmenting the proinflammatory effect of activated platelets.

The Role of Platelets in Hyperacute Rejection

Platelets contribute to hyperacute rejection of both allografts and xenografts. In hyperacute rejection, high titers of antibodies activate sufficient amount of complement to disrupt the endothelial cell monolayer and expose constituents of subendothelial matrix that are targets of platelet receptors such as GPIa and GPVI. Then conformationally active GPIIb/IIIa binds to fibrinogen, vWf, fibronectin and vitronectin. The combination of exposed subendothelial matrix, platelet phospholipids and tissue factor initiates the coagulation cascade. Activated platelets also promote tissue factor release from endothelial cells (22). Some of these interactions are intensified in discordant xenografts. For example, the participation of primate platelets in hyperacute rejection of porcine xenografts is accentuated by the increased interaction of human GPIb with porcine vWf (23).

When endothelial integrity is disrupted, the involvement of platelets and the coagulation cascade is obvious. However, Bustos et al. (24) explored more subtle interactions of xenogeneic platelets and endothelial cells. They found that unstimulated human platelets caused minimal changes in porcine endothelial cells, but platelets activated with small amounts of thrombin upregulated tissue factor activity, expression of E-selectin, and secretion of endothelin-1. Interleukin-1 (IL-1), a cytokine that platelets store in alpha-granules and can produce through splicing pre-mRNA into translatable RNA (25), participates in these endothelial responses.

P-selectin can augment interactions among endothelial cells, platelets and leukocytes in both allografts and xenografts. Rollins et al. (26) demonstrated that recombinant porcine P-selectin expressed either in a soluble form or on the surface of COS cells bound to human neutrophils. Similarly, human platelet-derived CD154 also has been shown to interact with CD40 on porcine endothelium and mediate activation (27).

Briscoe et al. (28) have shown that stimulation of endothelial cells through CD40 causes production of vascular endothelial growth factor (VEGF). VEGF, which was first described as vascular permeability factor by Dvorak et al. (29), causes vascular hyperpermeability, edema, exposure of tissue factor and deposition of fibrin. These effects are relevant to hyperacute and acute rejection.

Platelets in acute antibody-mediated rejection

Intravascular aggregates of platelets are prominent in experimental and clinical transplants that undergo acute antibody-mediated rejection (30-33). Meehan et al. (33) reported that over half of the biopsies from human renal transplants that meet the criteria for antibody-mediated rejection contained CD61⁺ platelet aggregates within peritubular capillaries. The intravascular platelet aggregates in cardiac allografts to mice and rats are covered with vWf (31,32).

Like P-selectin, vWf is stored in alpha-granules of platelets and Weibel-Palade bodies of endothelial cells (Table 1). However, new evidence indicates that there is heterogeneity in the contents of both alpha-granules in platelets and Weibel-Palade bodies in endothelial cells. In contrast to P-selectin, which most alpha-granules contain, only about half of alpha-granules contain vWf, and the other alphagranules contain VEGF, basic fibroblast growth factor and fibrinogen (34). This segregation of mediators into subpopulations of alpha-granules is evident in platelet precursors before they separate from megakaryocytes.

Endothelial cells are the likely source of the initial P-selectin and vWf released in antibody-mediated rejection because *in vitro* studies have demonstrated that the antibody-mediated cross

linking of MHC antigens releases contents of Weibel-Palade bodies within minutes (35). Antibody-mediated release of vWf does not require complement and no loss of cell viability occurs. This initial release of vWf can then engage platelets through several receptors including GPIb and GPIIb/IIIa.

In vivo studies have demonstrated that activation of complement can increase platelet interactions with endothelial cells. With the aid of C6-deficient donor and recipient rats, it was determined that the membrane attack complex (MAC) of complement increases vWf release from Weibel-Palade bodies and promotes intravascular platelet aggregates in arteries as well as capillaries of transplanted hearts and lungs (31,32). Similarly, reconstitution of immunoglobulin-deficient mice with monoclonal antibodies that activate complement is associated with the release of vWf and P-selectin from endothelial cells and aggregation of platelets in vessels of cardiac allografts (30).

Using a skin graft model that facilitates visualization of fluorescent-labeled platelets in real time, Morrell et al. have shown that alloantibodies induce increased interactions of platelets and leukocytes with endothelial cells as judged by decreased cell velocity in capillaries (36). Antibody-induced activation of platelets was evidenced by increased P-selectin and C3d on the surface of circulating platelets. Immunohistology of the skin grafts demonstrated increased release of vWf. Complement is not necessary for the antibody-induced interaction of platelets with endothelial cells in this model, but complement activating antibodies do induce more prolonged rolling of platelets and leukocytes. The retention of leukocytes by antibodies is in part dependent on platelets because platelet depletion abbreviates leukocyte rolling.

Platelets can augment antibody-induced vascular inflammation through multiple mechanisms, including activation of complement, expression of adhesion molecules, interactions of ligands and receptors, and release of chemokines, cytokines, nucleotides, serotonin and thromboxane as summarized previously (1). The interactions between platelets and monocytes or macrophages are relevant because these mononuclear cells are characteristic of antibody-mediated rejection. As shown in Figure 1, platelets attract mononuclear cells by secretion of chemokines, such as MIP-1 α (macrophage inflammatory protein; CCL3), RANTES (regulated upon activation, normal T cell expressed and secreted; CCL5), and MCP-3 (monocyte chemotactic protein; CCL7). After contact, monocytes and macrophages interact with platelets through receptor/ligand pairs, including P-selectin/PSGL-1 and CD40/CD154. Some of these same mechanisms also mediate interactions with T cells.

The Role of Platelets in Acute Cellular Rejection

In the absence of antibody-mediated rejection, activated platelets can promote leukocyte interactions integral to cell-mediated rejection. The expression of CD154 on activated platelets may be the most germane to cellular rejection. Initially shown to activate endothelial cells and promote endothelial cell expression of adhesion molecules (37), platelet-derived CD154 has now been shown to have direct stimulatory effects on most elements of alloimmune responses. Elzey et al. have shown that platelet-derived CD154 is sufficient to promote dendritic cell maturation, B-cell isotype switching, and protective CD8⁺ T-cell responses during viral infection in mice (38). Czapiga et al. have demonstrated that human platelet-derived CD154 or recombinant CD154 trimers directly activate resting human dendritic cells and that this can be interrupted with monoclonal antibodies specific for CD154 (39). Finally, Xu et al. have demonstrated in a mouse model of vascularized heterotopic heart transplantation that platelet-derived CD154 or purified CD154 trimers can induce allograft rejection independent of any other source of this molecule, restoring allograft rejection in CD154 knockout mice (40). Additionally, antibody-mediated blockade of platelet-derived CD154 prevents allostimulatory effects of platelets. Interestingly, CD154 expressing platelets infused at the

time of surgery are shown to traffic to the allograft and spleen and accelerate rejection, while platelets infused 30 days after engraftment fail to induce rejection. This group has proposed that trauma resulting from the surgical procedure itself promotes platelet-activation and that this is a critical step in mediating CD154 release and its effects on cell-mediated alloimmune responses.

The mechanisms by which CD154 influences adaptive immunity may extend beyond its interaction with CD40. Recent evidence suggests that CD154 can bind to other ligands and influence cellular immunity through distinct pathways. CD154 has been shown to bind to Mac-1 (41). Mac-1 is an integrin (CD11b/CD18) on monocytes, neutrophils and natural killer cells, which serves both as an adhesion molecule and as a complement receptor (CR3) for iC3b (42). Mac-1 ligation is known to facilitate leukocyte transendothelial diapedesis and initiate phagocytosis and cellular cytotoxicity. CD154 has been shown to induce and bind to Mac-1 facilitating macrophage migration into atheromatous lesions, promoting atherosclerotic vascular disease, vascular injury and neointimal formation (41,43). CD154 may also bind integrin receptors including $\alpha 5\beta 1$ and $\alpha \text{IIb}\beta 3$ and in doing so influence cell migration and activation in a broader context than previously suspected (44).

Activated platelets express and secrete many factors that can localize lymphocytes to an allograft. Release of small vasoactive molecules like adenine nucleotides (ADP and ATP), ionized calcium, histamine, serotonin and epinephrine from dense granules of activated platelets can promote leukocyte infiltration and stimulate vasoconstriction and leukocyte diapedesis. Alpha granules contain many chemokines that attract and activate macrophages and lymphocytes (45,46), such as MIP-1 α , RANTES, MCP-3 and PF4 (CXCL4). Platelet-derived ICAM-1, P-selectin, IL-1 IL-6 and GM-CSF are also released upon alpha degranulation and are known to promote migration and maturation of antigen-presenting cells (45,46). Reciprocally, monocyte-derived SDF-1 (stromal cell-derived factor; CXCL12), TARC (thymus and activation-regulated chemokine; CCL17), and MDC (macrophage-derived chemokine; CCL22) induce platelet activation, aggregation and adhesion after their binding to platelet receptors CXCR4 or CCR4 (47).

Potential platelet effects on chronic rejection

The vascular lesions of chronic rejection share some pathological features with both restenosis and atherosclerosis. Therefore, the many known contributions of platelets to both atherosclerosis and stenosis in animal models are potentially relevant to transplantation. The use of chimeric animals has been one fruitful strategy used to dissect the relative contribution of mediators that are produced by both platelets and endothelial cells to these processes (Table 1). Using bone marrow chimeras between normal and P-selectin knockout mice, Burger and Wagner (48) investigated the relative contribution of P-selectin from platelets and endothelial cells to the formation of atherosclerotic lesions in apolipoprotein E-deficient mice. In this model, endothelial cells produce the majority of circulating soluble P-selectin, and P-selectin expressed on endothelial cells was critical for the development of atherosclerotic lesions, but P-selectin on platelets promoted migration of smooth muscle cells into the lesion.

Wang et al. (49) also used bone marrow chimeras in a model of restenosis. They found that less neointima developed in normal mice reconstituted with bone marrow from P-selectin knockout donors than in the reverse chimera. Therefore, they concluded that expression of P-selectin on platelets, but not on endothelial cells, plays a crucial role in the development of neointimal formation after arterial injury. In addition, RANTES and MCP-1 were detected by immunohistology in the neointima. RANTES released by platelets has been shown to be immobilized on inflamed endothelium and causes arrest of monocytes on arteries (50).

Platelet-derived growth factor (PDGF) is another mediator of demonstrated importance to restenosis and arteriopathy that both platelets and endothelial cells are capable of secreting (Table 1). PDGF enhances neointimal formation in models of restenosis by stimulating migration, differentiation and proliferation of smooth muscle cells (51).

Various antagonists for receptors in platelets diminish inflammatory responses related to restenosis and arteriopathy. To dissect the role of platelets in promoting vascular injury, Konishi et al. (52) pretreated mice with soluble collagen receptor GPVI-Fc to inhibit adhesion of platelets to collagen exposed in injured carotid arteries. Pretreatment of mice with GPVI-Fc significantly reduced adhesion of dendritic cells immediately after inflicting vascular injury. In cardiac allografts, Wang et al. found that blockade of CD40 and CD154 decreased transplant arteriopathy (53). The immunohistology presented in the article demonstrated decreased intra-arterial platelet aggregates.

Clinical studies have correlated markers of platelet activation with various forms of arteriopathy. One study reported that increased neointimal thickness in coronary arteries of cardiac transplants was associated with increased expression of CD41 and GPIIb/IIIa on circulating platelets (54). Another study found a correlation between P-selectin expression on platelets and atherosclerotic lesions in carotid arteries (55). Atherosclerotic plaque is a known source of SDF-1, which can bind to and activate platelets through CXCR4 and CCR4. Activated platelets release PF4, PDGF and beta-thromboglobulin that promote fibroblast chemotaxis (56).

Clearly, the potential contribution of platelets to chronic vascular pathology in transplants deserves further investigation.

Potentials for platelet-directed therapy

The predominant dilemma in the inhibition of platelet-mediated immune stimulation is the elegantly efficient, interwoven nature of prothrombotic and proimmune functions of platelets. Most immunostimulatory molecules found in platelets to date are localized in the alpha granule, and alpha degranulation is a fundamental means of mediating prothrombotic effects of platelets. Thus, drugs directed toward platelet degranulation are likely to limit the salutary effects of platelets necessary for safe conduct of major surgical procedures. While complete platelet inhibition is not prudent, there may be a window in which clinicians can attenuate proimmune effects without inducing an overt coagulopathic platelet defect. That happy medium is yet to be defined.

Aspirin is currently the only drug routinely used to decrease platelet function in transplant recipients, mainly for its demonstrated efficacy in reducing the risk of acute coronary syndrome, but it has also been used to prevent hepatic artery thrombosis post liver transplantation (57). Polyclonal antithymocyte globulins are also used in a substantial number of transplant recipients and are known to induce a thrombocytopenia. Though typically deemed an undesirable side effect, thrombocytopenia may contribute to the therapeutic effect of polyclonal antibodies. Similarly, there are no data relating aspirin use to specific immune effects in humans, although aspirin has been related to improved allograft survival when used after renal transplantation (58,59). However, the cause of this effect is not clearly attributable to antiplatelet effects (60). Dipyridamole is also known to inhibit proinflammatory effects of platelets (61), but has not been systematically studied in transplantation.

Several mediators released by platelets are also present on other cell types, thus therapeutic intervention has not been clearly determined to be related to an antiplatelet effect. P-selectin glycoprotein ligand 1 (PSGL-1) is a ligand for P-selectin that facilitates platelet adhesion to endothelial cells and promotes platelet-mediated endothelial cell activation (62). It also binds

other selectin molecules including E-selectin and L-selectin. Use of a PSGL-1 antagonistic fusion protein, PSGL-1-Ig, has been shown to markedly attenuate the effects of ischemia reperfusion injury in transplant models (63). Similarly, CD154 and its receptor CD40, are broadly expressed on many cell types. Interruption of this pathway is clearly established as a means of attenuating experimental allograft rejection (reviewed in (64) and many agents have been developed to achieve this goal. Those targeting CD154 have been largely eschewed for fear of prothrombotic effect, however, the targeting of CD40 appears to be promising and is likely to be a source of new therapeutic intervention (65,66). Although the critical site of action remains undefined, it is likely that platelet-derived CD154 needs to be considered in clinical applications.

Purinic receptors have captured much attention particularly as related to the prevention of coronary thrombosis (67). Two prominent agents are available in this class, Ticlopidine and clopidogrel both have been shown to inhibit the P2Y₁₂ (12) receptor on platelets, an ADP receptor mediating platelet membrane stability and controlling platelet aggregation (68). Clopidogrel, has been used in transplantation particularly in patients who are allergic to aspirin. HUS, polyarthralgias and other complications have been reported without indications of immune benefits (69). These agents have known antithrombotic properties that are tolerable outside the surgical setting, but the risk of increased bleeding may limit their use in the immediate perioperative period. Recognizing the potential role of ADP inhibition in platelet function Robson et al. have developed methods to deliver soluble ectonucleotidases, particularly those of the CD39 family, that present a novel potential therapeutic strategy to limit vascular inflammation and prolong graft survival (20,70). Induction of CD39 on vascular endothelium by viral vectors has been shown to prolong graft survival in several models. This approach has the advantage of addressing platelet effects by targeting the endothelium and it may limit the risk of interfering with platelet aggregation or other prothrombotic activities. This remains an interesting area for development.

Another potential target for therapeutic intervention in platelet degranulation is serotonin (5-HT). Sarpogrelate, a 5-HT receptor antagonist, is currently used in Raynaud's syndrome and peripheral vasculopathy. It has recently been shown to extend the survival of fully mismatched heterotopic cardiac allografts in mice (71), and an anecdotal case report has suggested efficacy in a patient with chronic graft versus host disease (72). This pathway also remains an interesting avenue for exploration.

While specific inhibition of platelet effects remains an interesting area of development, there are at present no therapeutic antiplatelet regimens established for clinical use in transplantation. However, many precautions may be taken to avoid excessive platelet activation. Good surgical technique remains the most obvious means to avoid bleeding and transfusion. Judicious restraint of platelet transfusions at the time of surgery may prevent unnecessary immune stimulation. Platelet transfusions are a source of activated platelets and augmented soluble mediators. During storage platelets become partially activated as measured by P-selectin expression and soluble CD154 (73,74).

Conclusions

The data linking platelets to fundamental mechanisms of adaptive immunity are increasingly compelling. While alloimmune processes are undoubtedly redundant and complex, a critical role for platelets in allograft rejection appears likely, particularly in the setting of surgical transplantation. At present, platelet-associated immune activation is of mechanistic interest, although there are some immediate implications of this knowledge. Many pathways are available for therapeutic intervention, but the most fruitful ones will be those targeted in such a way so as to avoid interference with physiological platelet aggregation and hemostasis.

Specific antagonists to the end target of platelet-derived mediators (e.g. P2Y receptors, CD40 and serotonin) appear most promising for future development.

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Figure 1. Platelets expand interactions of monocytes/macrophages and T cells with endothelial cells through secretion of multiple chemokines and expression of ligands for receptors

Whereas, endothelial cells rapidly release stored chemokines for neutrophils (e.g. GRO-alpha and IL-8), platelets extend the array to include the release of stored chemokines for macrophages and T cells (e.g. MIP-1alpha, RANTES, MCP-3 and PF4). After macrophages and T cells localize to a vessel with activated platelets, cellular interactions are promulgated through receptor-ligand interactions. Activated platelets express P-selectin and CD40L (CD154). Macrophages express PSGL-1 and CD40. In addition to CD40, Mac-1 on macrophages can interact with CD154 on both platelets and T cells.

Table 1

Mediators common to endothelial cells and platelets

Category	Mediator	Endothelial cell	Platelet
Adhesion	vWf	In Weibel-Palade bodies	In some alpha-granules ⁴
	P-selectin	In Weibel-Palade bodies	In most alpha-granules ⁴
	PECAM ¹	Intracellular junction	Alpha-granule membrane protein
Chemokine	GRO- α (CXCL1) ²	Histamine-responsive vesicles ³	In alpha-granules
	IL-8 (CXCL8)	In Weibel-Palade bodies	In alpha-granules
Mitogen/activation	VEGF	Synthesized	In some alpha-granules ⁴
	bFGF	Synthesized	In some alpha-granules ⁴
	PAF	Synthesized; juxtacrine display ⁵	Juxtacrine display ⁵

¹ Homophilic interactions between platelet endothelial cells.

² GRO = growth-related oncogene.

³ Histamine-responsive vesicles that are smaller and distinct from Weibel-Palade bodies.

⁴ P-selectin is in most alpha-granules, but only about half of alpha-granules contain vWf and the other alpha-granules contain VEGF, basic fibroblast growth factor and fibrinogen (34).

⁵ Translocated to and displayed on the plasma membrane to interact with adjacent cells.